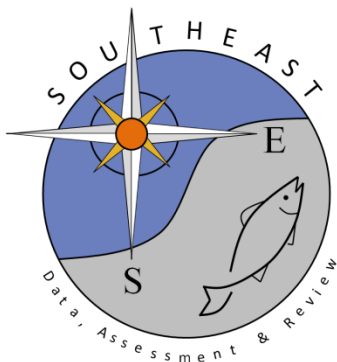


Application of the bomb radiocarbon chronometer with eye lens core
 $\Delta^{14}\text{C}$ for age validation in deepwater reef fishes

William F. Patterson, III and Derek W. Chamberlin

SEDAR92-RD-09

October 2024



This information is distributed solely for the purpose of pre-dissemination peer review. It does not represent and should not be construed to represent any agency determination or policy.

Application of the bomb radiocarbon chronometer with eye lens core $\Delta^{14}\text{C}$ for age validation in deepwater reef fishes

William F. Patterson, III  and Derek W. Chamberlin 

School of Forest, Fisheries, and Geomatics Sciences, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, USA

Corresponding author: William F. Patterson (email: will.patterson@ufl.edu)

Abstract

Eye lens core $\Delta^{14}\text{C}$ of Gulf of Mexico outer shelf and upper slope reef fishes matched a regional $\Delta^{14}\text{C}$ reference time series, but otolith cores were depleted in ^{14}C relative to the reference series. This is due to C in eye lens protein being derived from metabolic sources, with the ultimate source being epipelagic phytoplankton. In contrast, otolith C is derived mainly (70%–80%) from dissolved inorganic C, which is depleted in ^{14}C beyond the epipelagic. Analyzing $\Delta^{14}\text{C}$ in eye lens cores provides a novel approach for validating age estimates of deepwater (>200 m) fishes, which were unresolved in earlier applications of the bomb ^{14}C chronometer.

Key words: eye lens, radiocarbon, age validation, deepwater reef fish

Introduction

Bathybenthic and abyssal benthopelagic (hereafter, deepwater) reef fishes constitute important fishery resources in several regions of the globe, including US waters of the northern Gulf of Mexico (nGOM) and Atlantic Ocean (Atlantic). Many of these species have complex life histories, including hermaphroditism, great longevity (>100 years in some cases), and slow growth. However, estimating the fundamental parameter in population dynamics, age, has been elusive for many deepwater reef fishes due to difficulty in distinguishing otolith opaque zones, which may result in ageing bias or low precision among readers (e.g., Wakefield et al. 2017). Therefore, age validation is perhaps even more critical for deepwater reef fishes than ones found in shallower habitats (Andrews et al. 2013; Barnett et al. 2020). Unfortunately, chemically marking otoliths is not an option for deepwater fishes due to barotrauma, and radiometric approaches to age validation lack precision, with estimation error often being ± 10 years (e.g., Lombardi-Carlson and Andrews 2015).

The bomb radiocarbon (^{14}C) chronometer, which has been widely applied to validate age estimation in marine fishes, has also been applied in age validation studies of deepwater reef fishes in recent years. The principle behind this tool is the approximate doubling of atmospheric ^{14}C as a result of nuclear weapon testing in the 1950s and 1960s (Kalish 1993), with the signature of that rise, as well as the post-1970s decline being incorporated into the aragonite (biogenic CaCO_3) skeletons of hermatypic corals around the globe (Grottoli and

Eakin 2007). The coral bomb ^{14}C record has been utilized to examine a wide range of oceanographic and climate questions, but in fish ecology, its greatest utility has been as a reference time series for validating age estimation. For bony fishes, this typically involves sectioning one sagittae per individual for ageing, extracting the core of the opposite sagittae for ^{14}C analysis (typically reported as $\Delta^{14}\text{C}$), and then assessing the correspondence between otolith core (i.e., birth year) $\Delta^{14}\text{C}$ values and the reference $\Delta^{14}\text{C}$ time series specific to the ocean basin in which fish were sampled (Kalish 1993).

The bomb ^{14}C chronometer has been successfully employed to validate age estimation in several GOM reef fishes that occur on the continental shelf. However, its application for age validation in nGOM and Atlantic deepwater reef fishes has typically increased rather than reduced uncertainty in age estimates. For example, among a suite of nGOM or Atlantic deepwater reef fishes, including reef-associated pelagic barrelfish *Hyperoglyphe perciformis* ($n = 8$; Filer and Sedberry 2008), benthic golden tilefish *Lopholatilus chamaeleonticeps* ($n = 21$; Lombardi-Carlson and Andrews 2015), and benthopelagic yellowedge grouper *Hyporthodus flavolimbatus* ($n = 21$; Cook et al. 2009), there was a mean ($\pm 95\%$ confidence interval (CI)) difference of 68.5‰ ($\pm 5.26\%$) between the regional $\Delta^{14}\text{C}$ reference series reported by Barnett et al. (2018) and otolith core $\Delta^{14}\text{C}$ values. This is likely due to the fact that 70%–80% of otolith C is derived from dissolved inorganic carbon (DIC) (reviewed in Chung et al. 2019), and DIC becomes progressively depleted in ^{14}C relative below the epipelagic (Barnett et al. 2020).

Patterson et al. (2021) hypothesized that eye lens cores should be a better structure than otolith cores to measure birth year $\Delta^{14}\text{C}$ for deepwater fishes because lenses are derived from metabolic C instead of DIC, and nearly all of the organic C available to deepwater fishes is derived from phytoplankton that incorporate contemporary $\Delta^{14}\text{C}_{\text{DIC}}$ values into the organic C they fix. The objective of this study was to test this hypothesis by comparing eye lens and otolith core $\Delta^{14}\text{C}$ values for fishes sampled on outer continental shelf (150–200 m) and upper slope (to 400 m) reef habitats in the nGOM. The depth range of samples provides important context for interpreting differences between reef fish otolith and eye lens core $\Delta^{14}\text{C}$ values.

Methods

Reef fishes were sampled on the nGOM continental shelf or upper slope during 2015–2020 hook and line or bottom longline fishery-independent sampling, with depth of capture recorded for each individual. Species included barrelfish, benthic blackbelly rosefish *Helicolenus dactylopterus*, golden tilefish, benthopelagic snowy grouper *Hyporhodus niveatus*, and yellowedge grouper. Fish were measured to the nearest mm total length, and then both sagittal otoliths were extracted, rinsed with deionized water, and stored dry in coin envelopes. Both eyes were then removed and placed on ice in labeled plastic bags, or stored frozen at sea in a -20°C freezer. Upon return to the laboratory, eye samples were transferred to a -80°C freezer.

The left otolith from each fish was embedded in epoxy, and then a 0.4 mm transverse section was cut through its core with a low-speed diamond-bladed saw. Each thin section was secured to a microscope slide with a toluene-based mounting medium, and then covered with the same medium. Age was estimated by counting opaque zones in each otolith section under a dissecting microscope with transmitted light.

Right otoliths were utilized to extract cores (age-0 otolith material) for subsequent $\Delta^{14}\text{C}$ analysis. Each otolith was embedded in epoxy and a 1.5 mm transverse section, centered on the otolith's core, was cut with a low-speed diamond-bladed saw. Sections were rinsed with 1% ultrapure HNO_3 to remove any surface contamination that may have been introduced in the sectioning process, and then repeatedly flooded with $18.3\text{ M}\Omega\text{ cm}^{-1}$ polished water. Cleaned otolith sections were stored dry in acid-leached plastic cell wells.

Otolith core extraction was performed with a Micromill (New Wave Research, Fremont, CA, USA) computer-controlled precision drilling instrument. All materials used in the extraction or storage of otolith or eye lens cores, including the Micromill drilling bit, were baked in a muffle furnace at 500°C for 24 h to remove any residual petrocarbon from the manufacturing process. Each 1.5 mm otolith section was secured to a microscope slide with mounting medium, and then viewed on the Micromill's scope camera with reflected light. This enabled the first opaque zone to be observed and a milling pattern to be programmed that targeted the core (age-0) region. The Micromill stage was programmed to move in x , y , and z planes such that a

targeted mass of approximately 2 mg of otolith powder was extracted from the age-0 region of each sample. Extracted otolith powder was stored in borosilicate vials.

To extract eye lens cores, the right eye was first thawed, and then its lens was removed through an incision made in the cornea. Each lens was wrapped in aluminum foil and freeze-dried for 12 h. This caused the outer layers (laminae) of the lens to split and begin flaking while leaving the age-0 core intact. Outer laminae were peeled away with forceps, ultimately revealing the core, with the target lens core diameter being 1 mm, with a mass of approximately 0.7 mg. We had no age-0 fish among study species to base this target lens core size. However, Patterson et al. (2021) estimated 120 mm age-0 red snapper *Lutjanus campechanus* had a mean lens diameter of approximately 2 mm, and subsequent analysis demonstrated that 1 mm lens cores had a mass of approximately 0.7 mg. Therefore, we targeted 1 mm diameter cores in this study as a precaution not to extract lens protein from later years. Once extracted, cores were stored in borosilicate glass vials.

Eye lens and otolith cores were analyzed for $\Delta^{14}\text{C}$ with accelerator mass spectrometry (AMS) and for $\delta^{13}\text{C}$ with isotope ratio mass spectrometry at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at the Woods Hole Oceanographic Institution. The stable C isotope, ^{13}C , was measured as the delta value $\delta^{13}\text{C}$, which is computed as the ratio $^{13}\text{C}/^{12}\text{C}$ relative to a standard (Peedee belemnite). Radiocarbon (^{14}C) is reported as the delta value $\Delta^{14}\text{C}$, which represents the activity of a sample relative to a standard corrected for age and $\delta^{13}\text{C}$ (Stuiver and Polach 1977). Reef fish eye lens and otolith core $\Delta^{14}\text{C}$ values were plotted versus estimated birth year and overlaid on the regional coral and known-age otolith $\Delta^{14}\text{C}$ reference series (Barnett et al. 2018) to examine the correspondence of lens or otolith core $\Delta^{14}\text{C}$ values to reference values. Birth year was estimated as year of capture minus age estimated from otolith opaque zone count, and then 0.5 year was added to account for the fact that extracted eye lens or otolith core material was formed during the age-0 year, not just at the beginning of it. Lastly, a correlation analysis was computed in MS Excel to test whether there was a significant relationship between the difference in eye lens minus otolith core $\Delta^{14}\text{C}$ values (lens – otolith core $\Delta^{14}\text{C}$ differential) and sample depth.

Results

Otolith and eye lens core $\Delta^{14}\text{C}$ were measured for four individuals of each study species (Table 1). Age estimates ranged from 2 years for a 343 mm snowy grouper to 55 years for a 344 mm blackbelly rosefish. The mean mass \pm 95% CI was 2.02 ± 0.19 mg among otolith core samples, and 0.74 ± 0.05 mg among eye lens cores. Mean \pm 95% CI AMS measurement error (σ) was $3.06 \pm 0.22\%$ for otolith cores and $2.39 \pm 0.15\%$ for eye lens cores.

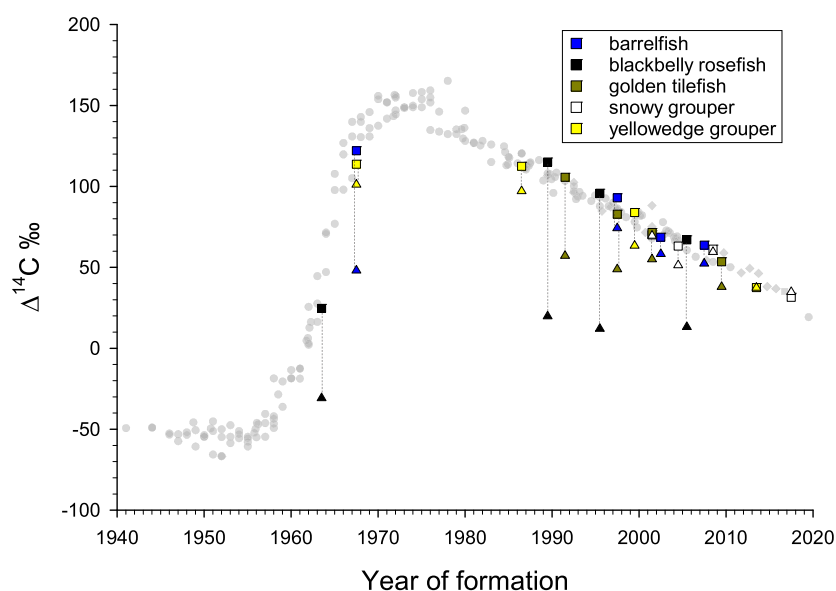
Eye lens core $\Delta^{14}\text{C}$ values corresponded well with the regional $\Delta^{14}\text{C}$ reference series among all study species, but otolith core $\Delta^{14}\text{C}$ values were consistently below the reference series data (Fig. 1). The mean \pm 95% CI lens – otolith core $\Delta^{14}\text{C}$ differential was lowest for snowy

Table 1. Descriptive data, eye lens core $\Delta^{14}\text{C}$, and otolith core $\Delta^{14}\text{C}$ for northern Gulf of Mexico (nGOM) outer shelf and upper slope reef samples analyzed with accelerator mass spectrometry (AMS) at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at Woods Hole Oceanographic Institution.

Species	Sample date	Depth (m)	Age (years)	Birth year	Total length (mm)	Lens core $\Delta^{14}\text{C}$ (‰)	Lens core σ (‰)	Otolith core $\Delta^{14}\text{C}$ (‰)	Otolith core σ (‰)	Lens minus otolith $\Delta^{14}\text{C}$ (‰)
Barrelfish	8 July 2017	351	15	2002	715	68.67	2.1	58.25	4.1	10.42
Barrelfish	8 July 2017	351	20	1997	779	92.99	2.1	74.25	3.4	18.74
Barrelfish	28 May 2020	364	53	1967	786	122.08	3.2	48.07	2.7	74.01
Barrelfish	28 May 2020	380	13	2007	605	63.76	2.2	52.37	4.0	11.39
Blackbelly rosefish	28 May 2020	364	15	2005	252	67.00	2.3	13.21	3.2	53.79
Blackbelly rosefish	28 May 2020	364	33	1987	344	115.09	2.9	19.80	2.6	95.29
Blackbelly rosefish	28 May 2020	380	25	1995	300	95.78	2.3	12.06	3.4	83.72
Blackbelly rosefish	28 May 2020	380	57	1963	408	24.66	2.4	-30.78	3.0	55.44
Golden tilefish	19 May 2020	230	11	2009	480	53.43	2.1	37.99	3.3	15.44
Golden tilefish	16 March 2015	287	18	1997	625	82.85	2.1	48.87	2.9	33.98
Golden tilefish	8 September 2020	292	19	2001	705	71.58	2.4	55.03	2.7	16.55
Golden tilefish	23 March 2015	379	24	1991	815	105.69	2.2	57.15	2.8	48.54
Snowy grouper	27 April 2019	36	2	2017	343	31.49	2.3	34.82	3.0	-3.33
Snowy grouper	8 September 2020	163	19	2001	771	70.11	2.4	69.62	3.6	0.49
Snowy grouper	29 May 2020	170	16	2004	864	63.12	2.3	51.34	3.1	11.78
Snowy grouper	9 July 2017	177	9	2008	707	61.23	2.5	59.52	2.2	1.71
Yellowedge grouper	15 March 2015	159	29	1986	846	112.42	2.2	97.06	3.1	15.36
Yellowedge grouper	28 May 2020	160	6	2014	562	37.52	2.9	37.40	2.6	0.12
Yellowedge grouper	30 May 2020	200	53	1967	999	113.72	2.3	101.16	2.7	12.56
Yellowedge grouper	24 March 2015	211	16	1999	742	83.87	2.1	63.43	2.9	20.44

Note: Age = opaque zone count in otolith sections. Species: barrelfish *Hyperglyphe perciformis*, blackbelly rosefish *Helicolenus dactylopterus*, golden tilefish *Lopholatilus chamaeleonticeps*, snowy grouper *Hyporthodus niveatus*, and yellowedge grouper *Hyporthodus flavolimbatus*.

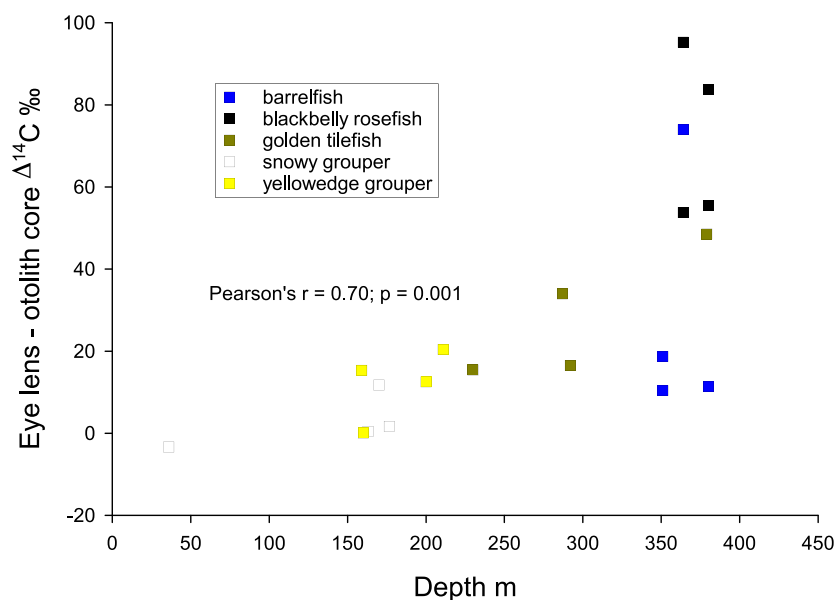
Fig. 1. Northern Gulf of Mexico (nGOM) reef fish eye lens (squares) and otolith (triangles) core $\Delta^{14}\text{C}$ values (linked by dashed lines) versus otolith-derived birth year estimates overlaid on the nGOM and Caribbean coral and known-age otolith $\Delta^{14}\text{C}$ reference time series. Legend indicates common names of fishes shown.



grouper ($2.26 \pm 10.26\text{‰}$), which were captured at depths between 36 and 177 m, and greatest for blackbelly rosefish ($91.54 \pm 51.93\text{‰}$), which were captured at 364–380 m (Fig. 1).

Overall, there was an increasing trend in the lens – otolith core $\Delta^{14}\text{C}$ differential with capture depth (Fig. 2), with a significant correlation existing between these parameters ($p = 0.001$; Pearson's $r = 0.70$). When barrelfish, which did

Fig. 2. Scatterplot of northern Gulf of Mexico (nGOM) reef fish eye lens core minus otolith core $\Delta^{14}\text{C}$ values versus depth of capture. Legend indicates common names of fishes shown.



not follow this general trend, were omitted from the analysis, the strength of the correlation increased ($p < 0.001$; Pearson's $r = 0.87$).

Discussion

Study results demonstrate the efficacy of utilizing eye lens cores when applying the bomb ^{14}C chronometer for age validation in outer shelf and upper slope reef fishes. While sample sizes were insufficient to conduct species-specific age validation analysis, eye lens core $\Delta^{14}\text{C}$ values among all species displayed close correspondence with the regional $\Delta^{14}\text{C}$ reference series that was first assembled by Andrews et al. (2013), and then extended by Barnett et al. (2018). This was in stark contrast to otolith core $\Delta^{14}\text{C}$ values, which were often substantially lower than eye lens core and regional reference series values.

Patterson et al. (2021) demonstrated the efficacy of utilizing eye lens core $\Delta^{14}\text{C}$ in bomb ^{14}C age validation applications, which was based on the lack of significant difference between otolith and eye lens core $\Delta^{14}\text{C}$ values for a suite of nGOM continental shelf reef fishes. They also hypothesized that eye lens core $\Delta^{14}\text{C}$ might be useful in age validation of deepwater reef fishes due to the fact that eye lenses are inert once formed, are composed of protein (crystallins) that is approximately 50% C by mass, and the protein is derived from metabolic C sources. This latter property is particularly important because the predominant basal endmember in the ocean is phytoplankton that fix organic C from DIC in the epipelagic (Broecker and Peng 1982). This organic C has a contemporary $\Delta^{14}\text{C}$ signature, which is then actively (vertical migrants) or passively (sinking particles) transported below the epipelagic on a daily time step via the oceanic biological

pump (Hernández-León et al. 2010). In contrast, DIC, which is the predominant source of C to accreting otoliths, is depleted in ^{14}C below the well-mixed surface layer (Barnett et al. 2020).

Barnett et al. (2020) reported a linear relationship existed between nGOM shelf edge (200 m) $\Delta^{14}\text{C}_{\text{DIC}}$, which had a value of approximately 40‰ during the time period of their samples and $\Delta^{14}\text{C}_{\text{DIC}}$ at 600 m, which was approximately -80 ‰. This trend of DIC being progressively depleted in ^{14}C between continental shelf edge and upper slope depths is reflected in otolith core values for outer shelf and upper slope fish samples in this study. The function reported by Barnett et al. (2020) predicts water column $\Delta^{14}\text{C}_{\text{DIC}}$ would be approximately -15 ‰ at 380 m, the greatest capture depth among nGOM reef fish samples in this study, but none of the blackbelly rosefish, golden tilefish, or barrelfish samples captured near that depth had otolith core $\Delta^{14}\text{C}$ values as low as -15 ‰, which may be explained by several potential factors. First, the percentage (20%–30%; Chung et al. 2019) of otolith C derived from metabolic sources, which would have had surface $\Delta^{14}\text{C}_{\text{DIC}}$ values (imparted via phytoplankton-fixed organic C), would raise otolith core $\Delta^{14}\text{C}$ above that of ambient $\Delta^{14}\text{C}_{\text{DIC}}$ at depth. Second, fish could have settled out of the plankton at shallower depths on the outer shelf or upper slope where $\Delta^{14}\text{C}_{\text{DIC}}$ values were higher, and then subsequently migrated to their depth of capture. Lastly, barrelfish, which were captured at similar depths as blackbelly rosefish but had otolith core $\Delta^{14}\text{C}$ values that were approximately 50‰ higher, are unique among study species in that they are pelagic versus benthopelagic or benthic. It is unknown at what depths age-0 barrelfish occur in the water column, or to what extent they migrate vertically to feed (Suca and Llopiz 2017). However, study authors have captured barrelfish in near-surface (>50 m) oceanic waters when sampling pelagic fishes around nGOM petroleum platforms, so it is likely that at least some

individuals vertically migrate well into the epipelagic to forage. This aspect of barrelfish ecology likely explains why the strength of the correlation between the lens – otolith core $\Delta^{14}\text{C}$ differential and capture depth increased when barrelfish were omitted from the model.

The general patterns observed in eye lens versus otolith core $\Delta^{14}\text{C}$ values among deepwater reef fishes in this study can be explained qualitatively based on what is generally known about the biology of these species and the distribution of $\Delta^{14}\text{C}_{\text{DIC}}$ in the nGOM water column. Moreover, the same pattern of deepwater reef fishes having otolith core $\Delta^{14}\text{C}$ values being substantially depleted in ^{14}C relative to regional $\Delta^{14}\text{C}$ reference series has been reported for deepwater reef fishes in other systems, including the Atlantic Ocean off the southeastern US (Filer and Sedberry 2008), the north Pacific Ocean off Alaska (Kastelle et al. 2008), and the tropical Pacific Ocean off Hawaii (Andrews and Scofield 2021). In general, these attempts at validating deepwater reef fish age estimates via the bomb ^{14}C chronometer have increased rather than reduced uncertainty in age estimates, although different approaches have been proposed to explain or control for the disparities between otolith core and reference series $\Delta^{14}\text{C}$ values. These include utilizing reference series from other regions than where samples were captured (Filer and Sedberry 2008), scaling otolith core $\Delta^{14}\text{C}$ values upward to better match the regional reference series (Kastelle et al. 2008), or disregarding some previously counted opaque zones in otolith sections such that otolith core $\Delta^{14}\text{C}$ better aligns with the regional reference $\Delta^{14}\text{C}$ time series (Andrews and Scofield 2021).

It is possible that original birth year estimates, hence otolith-derived ages, were more or less accurate in these earlier studies, and that uncertainty in age validation results arose principally from utilizing otolith core $\Delta^{14}\text{C}$ values in applying the bomb ^{14}C chronometer. Based on results presented here, it is likely that otoliths from deepwater fishes examined in previous studies recorded ^{14}C -depleted DIC signatures at depth, which did not match surface DIC values that exhibited the bomb ^{14}C signature. Perhaps deriving birth year $\Delta^{14}\text{C}$ signatures from eye lens cores, for the reasons stated above and the rationale provided in Patterson et al. (2021), would help resolve this issue. Clearly, that appears to be an effective approach for assessing the accuracy of age estimation in deepwater fishes from the nGOM, but the underlying principles should make it a useful in other ocean basins as well.

Acknowledgements

The authors thank Captain Johnny Greene and the crew of the *F/V Intimidator* for help in capturing samples, as well as Kristin Hannan and other NOAA Fisheries personnel onboard the Gulf of Mexico Bottom Longline Survey. The authors also thank Sue Handwork, Kathy Elder, and other scientists at NOSAMS for analyzing eye lens and otolith core samples for $\Delta^{14}\text{C}$. This work benefited from earlier discussions with Jeff Chanton, Beverly Barnett, Tom TinHan, Kate Overly, and Virginia Shervette about ^{14}C distribution in the ocean and its use in fish age validation.

Article information

History dates

Received: 4 January 2023

Accepted: 11 April 2023

Accepted manuscript online: 17 April 2023

Version of record online: 1 May 2023

Copyright

© 2023 The Authors. Permission for reuse (free in most cases) can be obtained from [creativecommons.org](https://creativecommons.org/licenses/by/4.0/).

Data availability

All data from this study are available within the manuscript.

Author information

Author ORCIDs

William F. Patterson, III <https://orcid.org/0000-0001-7588-7038>

0000-0001-7588-7038

Derek W. Chamberlin <https://orcid.org/0000-0002-4270-7245>

Author contributions

Conceptualization: WFP

Data curation: WFP, DWC

Formal analysis: WFP, DWC

Funding acquisition: WFP

Investigation: WFP, DWC

Methodology: WFP

Project administration: WFP

Resources: WFP

Supervision: WFP

Writing – original draft: WFP

Writing – review & editing: DWC

Competing interests

The authors declare no competing interests.

Funding information

Funding for this research was provided by the NOAA Fisheries Cooperative Research Program (awards NA18NMF4540080 and NA19NMF4540064 to WFP) and the University of Florida.

References

- Andrews, A.H., and Scofield, T.R. 2021. Early overcounting in otoliths: a case study of age and growth for gindai (*Pristipomoides zonatus*) using bomb ^{14}C dating. *Fish. Aquat. Sci.* **24**(1): 53–62. doi:[10.47853/FAS.2021.e6](https://doi.org/10.47853/FAS.2021.e6).
- Andrews, A.H., Barnett, B.K., Allman, R.J., Moyer, R.P., and Trowbridge, H.D. 2013. Great longevity of speckled hind (*Epinephelus drummond-hayi*), a deep-water grouper, with novel use of bomb radiocarbon dating in the Gulf of Mexico. *Can. J. Fish. Aquat. Sci.* **70**(8): 1131–1140. doi:[10.1139/cjfas-2012-0537](https://doi.org/10.1139/cjfas-2012-0537).
- Barnett, B.K., Thornton, L., Allman, R., Chanton, J.P., and Patterson, W.F., III 2018. Linear decline in red snapper (*Lutjanus campechanus*) otolith $\Delta^{14}\text{C}$ extends the utility of the bomb radiocarbon chronometer for fish age validation in the northern Gulf of Mexico. *ICES J. Mar. Sci.* **75**(5): 1664–1671. doi:[10.1093/ICESJMS/FSY043](https://doi.org/10.1093/ICESJMS/FSY043).
- Barnett, B.K., Chanton, J.P., Ahrens, R., Thornton, L., and Patterson, W.F., III. 2020. Life history of northern Gulf of Mexico Warsaw grouper *Hyporthodus nigritus* inferred from otolith radiocarbon

- analysis. PLoS ONE, **15**(1): e0228254. doi:[10.1371/journal.pone.0228254](https://doi.org/10.1371/journal.pone.0228254). PMID: [31978207](https://pubmed.ncbi.nlm.nih.gov/31978207/).
- Broecker, W.S., and Peng, T.-H. 1982. Tracers in the sea. Lamont Doherty Geological Observatory, Palisades, NY.
- Chung, M.-T., Trueman, C.N., Godiksen, J.A., and Gronkjaer, P. 2019. Otolith $\delta^{13}\text{C}$ values as a metabolic proxy: approaches and mechanical underpinnings. Mar. Freshw. Res. **70**: 1747–1756. doi:[10.1071/MF18317](https://doi.org/10.1071/MF18317).
- Cook, M., Fitzhugh, G.R., and Franks, J.S. 2009. Validation of yellowedge grouper, *Epinephelus flavolimbatus*, age using nuclear bomb-produced radiocarbon. Environ. Biol. Fish. **86**(4): 461–472. doi:[10.1007/s10641-009-9536-x](https://doi.org/10.1007/s10641-009-9536-x).
- Filer, K.R., and Sedberry, G.R. 2008. Age, growth and reproduction of the barrelfish *Hyperoglyphe perciformis* (Mitchill) in the western North Atlantic. J. Fish. Biol. **72**(4): 861–882. doi:[10.1111/j.1095-8649.2007.01761.x](https://doi.org/10.1111/j.1095-8649.2007.01761.x).
- Grottoli, A.G., and Eakin, C.M. 2007. A review of modern coral $\delta^{18}\text{O}$ and D^{14}C proxy records. Earth Sci. Rev. **81**(1-2): 67–91. doi:[10.1016/j.earscirev.2006.10.001](https://doi.org/10.1016/j.earscirev.2006.10.001).
- Hernández-León, S., Franchy, G., Moyano, M., Menéndez, I., Schmoker, C., and Putzeys, S. 2010. Carbon sequestration and zooplankton lunar cycles: could we be missing a major component of the biological pump? Limnol. Oceanogr. **55**(6): 2503–2512. doi:[10.4319/lo.2010.55.6.2503](https://doi.org/10.4319/lo.2010.55.6.2503).
- Kalish, J.M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet. Sci. Lett. **114**(4): 549–554. doi:[10.1016/0012-821X\(93\)90082-K](https://doi.org/10.1016/0012-821X(93)90082-K).
- Kastelle, C.R., Kimura, D.K., and Goetz, B.J. 2008. Bomb radiocarbon age validation of Pacific ocean perch (*Sebastes alutus*) using new statistical methods. Can. J. Fish. Aquat. Sci. **65**(6): 1110–1112. doi:[10.1139/F08-038](https://doi.org/10.1139/F08-038).
- Lombardi-Carlson, L.A., and Andrews, A.H. 2015. Age estimation and lead-radium dating of golden tilefish, *Lopholatilus chamaeleonticeps*. Environ. Biol. Fish. **98**: 1787–1801. doi:[10.1007/s10641-015-0398-0](https://doi.org/10.1007/s10641-015-0398-0).
- Patterson, W.F., III, Barnett, B.K., TinHan, T.C., and Lowerre-Barbieri, S.K. 2021. Eye lens $\Delta^{14}\text{C}$ validates otolith-derived age estimates of Gulf of Mexico reef fishes. Can. J. Fish. Aquat. Sci. **70**(8): 1131–1140. doi:[10.1139/cjfas-2020-0237](https://doi.org/10.1139/cjfas-2020-0237).
- Stuiver, M., and Polach, H.A. 1977. Discussion: reporting of ^{14}C data. Radiocarbon, **19**(3): 355–363. doi:[10.1017/S0033822200003672](https://doi.org/10.1017/S0033822200003672).
- Suca, J.J., and Llopiz, J.K. 2017. Trophic ecology of barrelfish (*Hyperoglyphe perciformis*) in oceanic waters of southeast Florida. Bull. Mar. Sci. **93**(4): 987–996. doi:[10.5343/bms.2017.1003](https://doi.org/10.5343/bms.2017.1003).
- Wakefield, C.B., O'Malley, J.M., Williams, A.J., Taylor, B.M., Nichols, R.S., Halafihi, T., et al. 2017. Ageing bias and precision for deep-water snappers: evaluating nascent otolith preparation methods using novel multivariate comparisons among readers and growth parameter estimates. ICES J. Mar. Sci. **74**(1): 193–203. doi:[10.1093/icesjms/fsw162](https://doi.org/10.1093/icesjms/fsw162).